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## Molecular characterization of by-products of saffron (*Crocus sativus* L.) production

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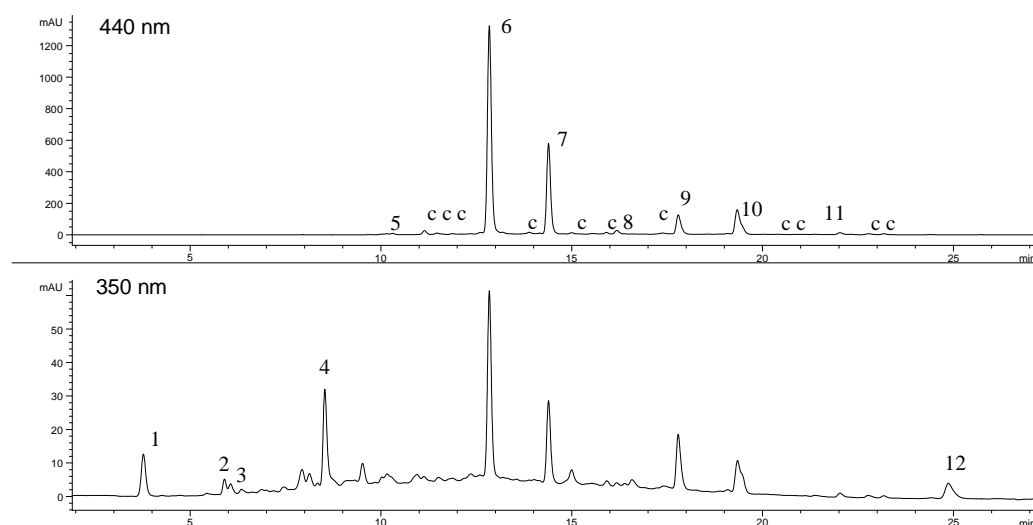
**Abstract.** Three different tissues, stigma, stamens and sepals, of *Crocus sativus* L. samples from two different geographic origins, were analysed for their crocins and flavonols content. The identification of crocins, safranal, picrocrocin, and flavonols was carried out by HPLC/DAD and HPLC/MS analysis. The stigma analysis showed that both samples, grown under natural conditions, exhibited high crocins content (between 342 and 231 mg/g), stamens and sepals are rich in flavonols (between 6 and 10 mg/g). While stamens contain mainly kaempferol derivatives, the sepals main flavonols were quercetin and methyl-quercetin glycosides. These data may be useful in order to find a possible exploitation of the by-products, which represent a very large percentage of the *Crocus sativus* L. flowers.

**Introduction.** The dried stigmas of *Crocus sativus* L. are a very expensive spice known as saffron used as food flavouring and colouring agent and as traditional herbal medicine. Crocus is cultivated in India, Iran, Spain, Greece and Italy. Many papers deal with analytical aspects to set up methods for the separation and determination of the biological active components [1-3], and aroma components [4-6]. The purpose of this paper is the analysis of stigmas from *Crocus sativus* cultivated in Italy (Perugia and Fiesole) to characterize these commercial saffron from a quality point of view and the characterization of the biological active components of stamens and sepals to find a possible use of this recycling material which is the most consistent part of *Crocus* flowers. The exploitation of stamens and sepals, notwithstanding their availability as by-products, has not been taken into account, with the exception of one paper dealing with the isolation of flavonoids from petals to study their tyrosinase inhibition action [7]. Notwithstanding the lack of information on the polyphenol content of these tissues, petal extracts were used to control rat blood pressure [8] and to test their antitussive effect in guinea pigs [9].

**Materials and methods.** Sepals, stamen and dried stigma sample were obtained from plants harvested in 2005 from Fiesole (FI, Italy) and Perugia (PG, Italy). Sepals and stamen (500 mg) were suspended in 50 mL of 70% ethanol, (pH 2.0 by HCOOH), filtered, evaporated and then redissolved in EtOH/H<sub>2</sub>O. Saffron stigmas (50 mg) were extracted with 10 mL of 70% ethanol and then filtered. These extracts were analysed by HPLC/DAD/MS for the determination of saffron components. Analysis for flavonols and crocins were carried out using a HP 1100L liquid chromatograph equipped with a DAD and a HP 1100 MSD mass spectrometer (API/electrospray interface, Agilent Technologies, Palo Alto, CA, USA). Flavonols and crocins were separated by using a 150 × 3.9 mm i.d. 4 µm Nova-Pak C18 column (Waters). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 308, 350 and 440 nm. The mobile phase was a one-step linear solvent gradient system, starting from 90% H<sub>2</sub>O (pH 3.2 by HCOOH) up to 100% CH<sub>3</sub>CN during a 60-min period, flow 0.8 mL min<sup>-1</sup>. The mass spectrometer was operated in positive mode at 120 eV. Quantification of individual compounds was directly performed by HPLC/DAD using a five-point regression curve ( $r^2 \geq 0.998$ ) on the basis of authentic standards. In particular, crocin derivatives were determined at 440 nm using curcumin as reference compound; safranal at 308 nm using safranal as reference compound and picrocrocin at 250 nm using p-OH-benzoic acid as reference compound. Flavonols, like kaempferol and quercetin derivatives, were determined at 350 nm using kaempferol-3 -glucoside and rutin, respectively, as reference. In all cases, at concentrations of single derivatives were calculated applying corrections of molecular weight.

**Results and Discussions.** The major biologically active components of saffron are crocin analogues which are all glycosides of *trans*-crocin, a carotenoid derivative which are responsible for colour. Safranal which is responsible for the characteristic aroma of saffron, is formed during the storage by dehydration of picrocrocin which is responsible for its bitter taste. Flavonoids are found in stigma,

sepals, and stamens. In Figure 1 there is reported a chromatogram of the stigmas extract recorded at two wavelengths (440 and 350 nm). The peak identification was performed through the recording of UV and MS spectra, as previously reported [3]. As regards stigma, the composition of the extract is similar to that found by other authors regarding crocins, picrocrocins, and safranal.



**Figure 1.** Chromatographic profile, acquired at 440 nm and 350 nm, of an hydroalcoholic extract from saffron stigmas at relative maximum of absorbance of crocins and flavonoids. Identified compounds: 1) kaempferol-3-sophoroside-7-glucoside, 2) kaempferol -3,7,4'-triglucoside, 3) picrocrocine, 4) kaempferol-3-sophoroside, 5) trans-crocine5, 6) trans- crocine4, 7) trans-crocine3, 8) trans-crocine2', 9) cis-crocine4, 10) trans-crocine2, 11) cis-crocine1, 12) safranal, C= crocine derivatives

Kaempferol derivatives were identified according to previous findings [3, 10]. In the case of stamens a lesser amount of crocins was found and other than kaempferol even quercetin derivatives were found. There are no differences, from a qualitative point of view between the two sampling zones; in fact only a quantitative variation was found in the case of samples from different geographic origins [10]. In Table 1 there are reported the quantitative data of dried stigma. The crocins content of the two samples is quite high giving evidence to the very good quality of the two samples. The crocins contents of stamens and sepals ranged from 4.2 to 196.3 µg/g while flavonols ranged from 6.06 to 10.14 mg/g. The flavonols composition of the two tissues is different: in sepals kaempferol derivatives ranged between 91 -93 %, in stamens quercetin and methyl-quercetin derivatives ranged between 52-71%.

Compounds	Stigma (FI)	Stigma (PG)
<b>Crocins</b>	342.48	230.76
<b>Picrocrocine</b>	111.10	68.94
<b>Safranal</b>	2.23	2.57
<b>Flavonoids</b>	12.13	9.64

From all these data the possible exploitation of alternative tissues like stamens and sepals as phytochemical resource can be pointed out. For each kg of stigma about 1000 kg of flowers are processed; therefore sepals and stamens are important by-product of saffron production and their use could increase the *Crocus sativus* flowers economic value.

**Table 1.** Data are expressed as mg/g sample. Data are the mean of three determinations (standard deviation < 3%)

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